REMARKS

Status of the Claims:

With the above amendments, claims 21 and 24 are amended and claims 14-26 are pending and ready for further action on the merits. No new matter has been added by way of the above amendments. Reconsideration is respectfully requested in light of the following remarks.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claim 15 has been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Examiner asserts that although the claims as filed are part of the specification, that the subject matter of claim 15 should be added to the written description. Applicants point out that support for claim 15 occurs at page 6, lines 3-9. Withdrawal of the rejection is warranted and respectfully requested.

Claims 21-24 have been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Examiner asserts because claim 21 recites a "method for the quantitative detection of 25-hydroxy- and 1α,25-dihydroxy vitamin metabolite . . .", it is unknown if the method should recite "or" instead

of "and". Claim 21 and claim 24 have been amended to recite "or" as suggested by the Examiner. It is believed that with this amendment that the rejection has been obviated. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 21-24 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Holick '127 (WO 97/24127).

This rejection is again traversed for the following reasons.

The invention of Holick '127 is not enabled as will be evident from the following discussion.

The instant invention relates to a method for detecting a vitamin D analogue that contains a hydroxyl group at the 25 position. Holick '127 does not disclose all of the elements of the instantly claimed invention.

First, for the Examiner's benefit, the attachment that the Examiner was indicated was missing from the response of August 7, 2002 showing the ring structures of the compounds of Holick '127 and the instant invention is attached. As was pointed out

in that response, the "biotin conjugate" shown on page 17 in Holick '127 is not biotin.

Second, the Examiner asserts that Figure 6 in Holick '127 discloses the correct ring structure for biotin. Applicants acknowledge that the compound displayed in Figure 6 has the correct ring structure for biotin but the intervening chain is a long ester to a 25-OH vitamin D moiety. The chemical formula shows an ester and the chemical reaction shown in Figure 6 produces an ester. As explained on page 7, lines 4 to 14 of the instant invention, human blood serum and plasma contain esterases, which attack ester chains such as is shown in Figure 6 in Holick '127.

Third, Applicants assert that the NMR and UV data present in Holick '127 merely shows that there was a vitamin D ring system present in its disclosure but there is no proof that the 25 OH-group was present in any one of the target compounds. In other words, the disclosure in Holick '127 is not enabled. The previous publications cited by the Examiner (Ray et al., Steroids, Vol. 60(8), pp.530-533 (1995) and Swamy et al., Protein Expression and Purification, Vol. 6(2), pp. 185-188, (1995)) allegedly describe the aminopropylation of 25 hydroxy

vitamin D. However, the relevant starting material 25-hydroxyvitamin-D_3-3α-3-aminopropylether (compound 4a) was never obtained and used in any one of the cited publications.

The newly recited references both refer to a method of synthesis described by Ray et al. in Biochemistry Vol. 30, pp. 4809-4813 (1991), which has been cited in the instant application. In the Ray et al. reference, cyanoethylated 25-OH vitamin D is reduced to the corresponding amine by means of $LiAlH_4$-$AlCl_3$ (Note that Ray et al. is cited in Cite 8 in Swamy et al. and Cite 5 in Roy et al.). However, the reduction of cyanoethylated 25-OH vitamin D by $LiALH_4$-$AlCl_3$ not only reduces the cyano group but also reduces the 25-hydroxyl group of the vitamin D if it is not protected as claimed in the instant invention. Thus, it appears that the reduction of the 25-hydroxyl group by $LiAlH_4$ was apparently not noted by Ray et al. This is proven by the fact that Holick '127 requires an eleven-fold surplus of biotinylated target compound to replace one molecule of 25-OH vitamin D on the vitamin D binding protein.

In other words, Holick '127 used, in its displacement studies, biotinylated vitamin D instead of biotinylated 25 OH-vitamin D. Thus, all of the binding and ELISA studies in Holick

'127 were made with derivatives of vitamin-D rather than 25 OH-vitamin D. There is no proof in Roy et al. or Swamy et al. that the 25-OH group was present in any one of the synthesized vitamin D derivatives. No mass spectrum was reported to prove that the target compound had indeed the calculated molecular weight, no IR data showing the O-H stretch is reported, no elemental analysis was reported showing the composition was reported, and the H-NMR data are all not conclusive as was pointed out in the response of August 7, 2002. Thus, it is maintained that neither Holick '127 nor any of Ray's previous publications contain an enabling disclosure. Holick '127 did not make biotinylated 25 OH-vitamin D as it asserts. The structural analyses of Holick '127 are useless in the regard that there is no showing that they made biotinylated 25 OH-vitamin D. The biological tests, in contrast, show that Holick '127 did not make biotinylated 25 OH-vitamin D because Holick '127 requires an eleven-fold increase in binding tests between vitamin-D (having no 25-hydroxyl group) and human plasma vitamin-D-binding protein.

The Scatchard plots submitted in the last response as well as the chemical formulas submitted in the last response are

again submitted herewith. In the Scatchard plots, the displacement efficiencies of 25-hydroxy vitamin D (as claimed in claims 25 or 26), a vitamin D dimer (as claimed in claims 25 or 26), and ^{3}H-25-OH-Vitamin-D on vitamin D binding protein from goat serum are shown. Please see example 3, (iv), line 18 et seq. on page 24 in the written description for the experimental protocol followed. As can be seen in the Scatchard plots, the displacement efficiencies of the compounds of the instant invention were all close to 1, whereas compound C in Holick '127 could only displace the corresponding tritiated compound from human vitamin D binding protein when it was present at an eleven-fold excess. In other words, compound C in Holick '127 is not the 25-OH-vitamin D compound. The disclosure of Holick '127 is not enabled for making this compound. The rejection is inapposite. Withdrawal of the rejection is warranted and respectfully requested.

Allowable Subject Matter

Applicants would like to thank the Examiner for acknowledging that claims 14, 16-20 and 25-26 are allowable.

Conclusion

With the above remarks and amendments, it is believed that the claims, as they now stand, define patentable subject matter such that a passage of the instant invention to allowance is warranted. A Notice to that effect is earnestly solicited.

If any questions remain regarding the above matters, please contact Applicant's representative, T. Benjamin Schroeder (Reg. No. 50,990), in the Washington metropolitan area at the phone number listed below.

Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicants hereby petition for an extension of one (1) month to February 28, 2003 in which to file a reply to the Office Action. The required fee of $55.00 is enclosed herewith.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any

additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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DRN/TBS/bsh
Attachments: Version with Markings to Show Changes Made
Scatchard Plot
Drawing of Biotin and Conjugate

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

The claims have been amended as follows.

21. (Amended) Method for the quantitative detection of 25-hydroxy- [and] or 1α,25-dihydroxy vitamin D metabolite in a sample, characterized in that a vitamin D derivative is obtained with a method according to claim 14, and is employed as a binding partner.

24. (Twice Amended) Reagent kit for the detection of 25-hydroxy- [and] or 1α,25-dihydroxy vitamin D metabolites, characterized in that it contains a standardized quantity of solid or solution of a vitamin D-derivative which is manufactured in accordance with claim[s] 14.

Figs. 8, 9 und 10 Schemadarstellungen kompetitiver, radioaktiver IRMAs für 25-OH-Vitamin-D mit Hilfe des erfindungsgemäßen 25-OH-Vitamin-D-Konjugats;

Figs. 11 und 12 Schemadarstellungen kompetitiver ELISAs unter Verwendung von Mikropartikeln;

Fig. 13 Schemadarstellung eines kompetitiven Bindungsassays für 25-OH-Vitamin-D mit Hilfe eines 25-OH-Vitamin-D-Konjugats gemäß der Erfindung und einem direktmarkierten Vitamin-D-bindenden Protein.

Fig. 14 ein Blockdiagramm des Vergleichs der 1,25-Dihydroxy-Vitamin-D-Gehalte in Seren von Dialyse- und Normalpatienten.

Fig. 1 zeigt den erfindungsgemäßen Syntheseweg zur Herstellung des bifunktionellen 25-OH-Vitamin-D-Konjugats. Zunächst wird 25-OH-Vitamin-D in einem Gemisch aus Acetonitril, Kaliumhydrid und tert.-Butanol mit Acrylnitril cyanoethyliert. Durch die Gegenwart des als Base fungierenden Kaliumhydrids und durch die Anwesenheit von tert.-Butanol zur Abwendung unspezifischer Reaktionen an der 25-Hydroxygruppe wird erreicht, dass selektiv die 3-Hydroxy-Gruppe des Vitamin-D cyanoethyliert wird. Die Ausbeute an 25-OH-Vitamin-D-3β-cyanoethylether beträgt in der Regel etwa 74% bei einer Reaktionsdauer von 40 Minuten.

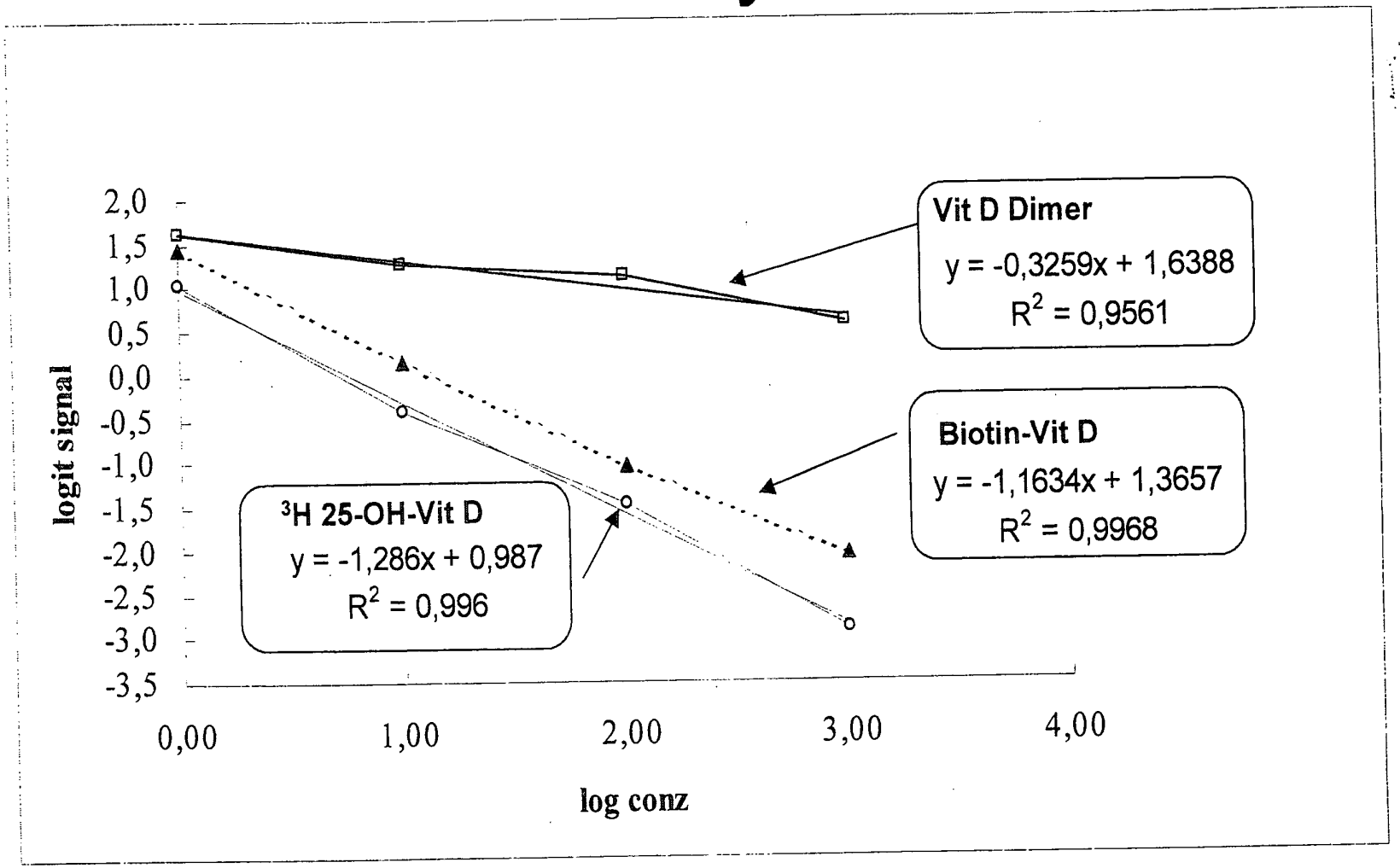
Nach üblicher Aufarbeitung wird der 25-OH-Vitamin-D-3β-cyanoethylether mit Lithiumhydrid versetzt und die 25-Hydroxygruppe in das Lithiumalkoholat überführt. Es folgt eine Reduktion des Nitrils mit $LiAlH_4$ zu 25-OH-Vitamin-D-3β-3'-aminopropylether. Dieser Schritt ist quantitativ, ohne dass Nebenprodukte auftreten. Zuletzt erfolgt gegebenenfalls eine Biotinylierung mit einem aktivierten Biotinylierungsreagenz wie LC-BHNS (Biotinyl-N-ε-aminocaproyl-hydroxy-succinimidester). Die resultierende Abstandsgruppe X hat entsprechend der Aminocaproylkette eine Länge von etwa 0.8 bis 0.9 nm.

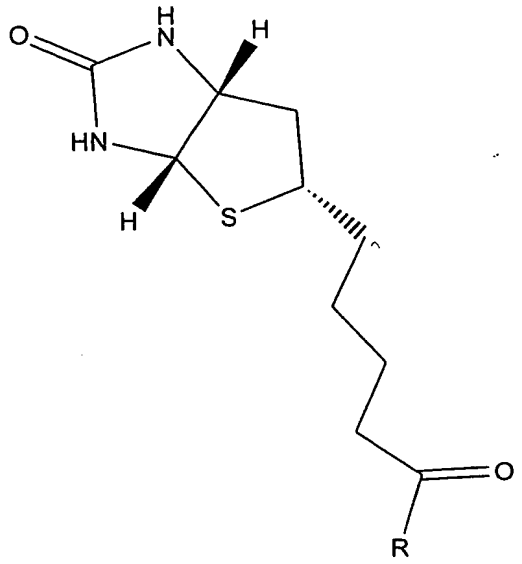
25-OH-Vitamin-D-3β-3'[6-N-(biotinyl)hexamido]amidopropylether ist temperaturstabil und kann in einer wässrigen, leicht sauren Matrix über mehrere Monate gelagert werden. Da die Verbindung von Serumenzymen nicht gespalten wird, eignet sie sich bestens für diagnostische Routinetests in Serum, Plasma und Urin.

Fig. 2 zeigt eine Schemadarstellung eines kompetitiven ELISA für 25-OH-Vitamin-D. Hierbei wird das 25-OH-Vitamin-D-Konjugat (25-OH-Vitamin-D-3β-3'[6-N-(biotinyl)hexamido]amidopropylether) über Streptavidin an eine feste Phase gebunden. Dann erfolgt in flüssiger Phase die kompetitive Bindung von Vitamin-D-bindenden Protein und 25-OH-Vitamin-D aus Standard oder Probe an das 25-OH-Vitamin-D-Konjugat. Der Nachweis erfolgt durch Peroxidase-markierte Antikörper gegen das Vitamin-D-bindende Protein. Der Fachmann weiß, dass auch andere Markerenzyme verwendet werden können, zum Beispiel alkalische Phosphatase oder Galaktosidase, etc.

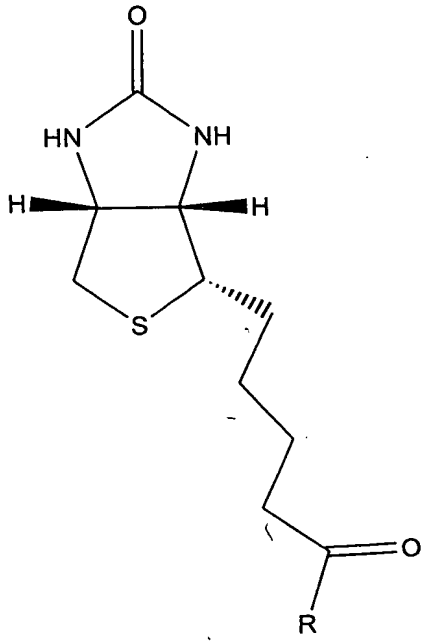
Fig. 3 zeigt die Schemadarstellung eines kompetitiven, nicht-radioaktiven ELISA, wobei das Vitamin-D-bindende Protein zunächst über anti-Vitamin-D-Bindungsprotein-Antikörper an die feste Phase gebunden wird. Danach erfolgt in flüssiger Phase die kompetitive Bindung von 25-OH-Vitamin-D-Biotin und 25-OH-Vitamin-D aus Standard bzw. Probe. Zur Bestimmung wird dann

Bioactivity Vit D





Biotin group as used (shown in examples) by Holick et al. in WO 97/24127 or US 5,981,779



Biotin group of the present invention